Papers and Originals

The Endothelial Cell*

LORD FLOREY, O.M., M.A., M.D., PH.D., F.R.S.

[WITH SPECIAL PLATE]

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It was with very great pleasure that I accepted the kind invitation to give a lecture in honour of my old friend, Sir Roy Cameron, who has done so much for this College, and by his outstanding teaching and research has forwarded the interests of pathology throughout the world. We not only respect him as a great pathologist but admire his wide historical erudition.

It was natural, while I was thinking of what I should say today, for me to recall with admiration—and, let me add, astonishment—that one man had been able to write such a monumental work as *The Pathology of the Cell*. In this he displayed his virtuosity, not only in the way he presented his knowledge of the cell, but also in the way he brought out the very interesting and exciting sweep of the growth, over recent centuries, of our knowledge of the enormously complex activities of cells. Unfortunately nearly all of us now have to concentrate on a very narrow field if we are to acquire a detailed understanding of a subject, and this applies particularly to me.

Cameron's great interest in the cell suggested that today I might appropriately attempt a brief review of our current knowledge of the endothelial cell, knowledge which, like that of many other cells, has greatly increased in the past ten to twelve years. From the morphological point of view this is largely due to the use of the electron microscope.

As is generally known, a so-called "typical endothelial cell" is a flattened cell which is about 3 microns thick in the neighbourhood of the nucleus but which thins out to 0.2 micron or less at the periphery of the cell. The cells are usually elongated, being some 30 microns long by 10 microns wide. These flat nucleated cells are joined to one another to form the innermost lining of both blood and lymphatic channels. In order to keep the subject within bounds I shall deal only with mammalian cells, principally those from the rat and the mouse.

A few years ago the endothelial cell was thought of as a structure so thin that few details could be made out in its cytoplasm, while now it is recognized that there are many kinds of endothelial cell which differ from one another substantially in structure, and, to some extent, in function. I propose to look at the main types of cell, and the organelles which can be seen in them, and then to discuss briefly what we know of the function of some of the structures.

Types of Endothelial Cell

A number of types of endothelial cell have been described. Those found in cardiac muscle, skeletal muscle, and subcutaneous tissue have cytoplasm which is relatively uniform in thickness. Endothelial cells in the pancreas, intestine, pituitary gland, and some other organs have "pores" or areas of extreme thinness which are composed of the plasma membrane or even possibly the outer leaflet only of what is known as a unit membrane. The endothelial cells of the lung have some very tenuous areas, which are contained between two plasma membranes, as well as somewhat thicker areas. The sinusoids of the liver are lined by Kupffer cells which are prolonged into tenuous portions that are joined to neighbouring endothelial cells by recognizable intercellular junctions.

The endothelium of the cerebral capillaries is closely invested by neuroglia, and is remarkable in that it contains very few of the caveolae intracellulares which are so prominent in the capillaries of cardiac muscle.

The main structures that can be seen in the endothelial cells of the above types are illustrated in the figures (see Special Plate), and for remarks about them the reader is referred to the appropriate legends.

It is probably reasonable to say that the functions of the organelles such as the mitochondria and the Golgi apparatus do not differ substantially from those performed by them in other cells. It is probable that the endoplasmic reticulum and ribosomes, as in other cells, are engaged in manufacturing proteins and enzymes not only for the internal use of the cell but also some, such as clearing factor lipase and pro-activator of fibrinolysin, which escape from the cell. Nothing definite can be stated about the function of dense bodies (Special Plate, Fig. 8) nor of the recently described Weibel-Palade bodies (Fig. 7).

Although little can be said about the multivesicular bodies found in endothelial cells (Figs. 4 and 5) it seems to be certain that if particles such as molecules of ferritin and saccharated iron oxide are injected into the blood-stream some find their way into them in all the endothelium so far examined (Fig. 6). How these marker particles reach the multivesicular bodies is unknown, nor has their ultimate fate been determined.

Passage Through the Endothelial Barrier

The processes by which substances pass the endothelial barrier which is interposed between the intravascular and extravascular spaces have occupied the attention of physiologists for very many years. In what follows only the passage of large molecules or small particles will be considered. The most plausible and well-supported physiological theory demands the presence of pores in capillaries which have been estimated to have a radius of 30–45 Å. The recognition of structures of this size is well

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LEGENDS TO SPECIAL PLATE

FIG. 1.—Endothelial cells of cardiac capillary. In this picture parts of two endothelial cells are shown. The junctions between them are narrow slits which appear darker than the surrounding cytoplasm. The basement membrane is closely applied to the cells and is similar to that covering the neighbouring cardiac muscle. The cytoplasm contains many vesicles. (×16,000.)

FIG. 2.—This picture shows portions of three endothelial cells from cardiac muscle. The space at the junctions between them can be seen to vary in width. At the constricted portions the cytoplasm is darker. These portions are beginning to be known as tight junctions. Mitochondria (M), ribosomes (R), and endoplasmic reticulum (ER) can be seen. Caveolae intracellulares are present on both luminal and external surfaces of the cells. A portion of an adventitial cell is seen. (×32,000.)

FIG. 3.—Endothelial cell from aorta. This picture is to show the Golgi body, which is composed of the usual parallel laminated structures and vesicles. (×23,600.)

FIG. 4.—Two multivesicular bodies in two endothelial cells of the pancreas. (×32,000.)

FIG. 5.—One of the multivesicular bodies from Fig. 4. It shows that the membranes bounding the main body and around the small vesicles inside it are of the unit membrane type—that is, composed of two outer dark layers with a less dense intermediate zone. (×170,000.)

FIG. 6.—Two multivesicular bodies in endothelium from the small intestine. They contain many molecules of ferritin which was injected into the blood-stream. Some of the ferritin can be seen free in the lumen of the vessel. Some ferritin is also seen in caveolae intracellulares—for example, at upper arrow. (×73,500.)

FIG. 7.—Aortic endothelium. Bodies similar to this were described by Weibel and Palade. They apparently consist of parallel tubes closely joined together. One body is cut longitudinally and another tangentially. Their function is unknown. (×73,500.)

FIG. 8.—A "dense" body in endothelium of a pancreatic capillary. The fine particles in the lumen are ferritin molecules. (×55,000.)

FIG. 9.—Centrioles in aortic endothelium. One is cut transversely and another longitudinally. They are seen to consist of parallel tubes. (×63,000.)

FIG. 10.—Endothelium of cardiac capillary. This is particularly to show the caveolae intracellulares. They appear to be infoldings of the plasma membrane of the cell. They are on both the luminal and external surface of the cell. In some places they appear to touch one another—for example, at x—and some appear almost to span the full width of the cell (y). At z there may be some vesicles which are isolated in the cell, but this appearance may be due to the angle at which the section is cut. (×49,500.)

FIG. 11.—In this section the endothelial cell is cut obliquely near a surface. It shows that the caveolae intracellulares are not always simple invaginations but that they may join one another to form clusters. The pale areas seen in some vesicles—for example, at x—are the mouths of caveolae sectioned transversely at the cell surface. (×77,000.)

FIG. 12.—A caveola abutting on the external surface of a pancreatic endothelial cell. Shows that its lining membrane is of unit structure and is continuous with the plasma membrane of the cell surface. (×170,000.)

FIG. 13.—A collection of "coated vesicles" from endothelial cells of a number of organs. These coated vesicles (arrows) are more prominent and larger than the usual caveolae intracellulares and associated vesicles. They have a coat of fine "bristles" and appear to have a dense lining. (×36,000.)

FIG. 14.—"Flaps" on the internal surface of endothelial cells. They occur particularly near junctions between endothelial cells, but they are to be found elsewhere. They may be associated with the ingestion of large particles, but their function is unknown. (×27,600.)

FIG. 15.—A junction between two endothelial cells. The space between the cells is filled with some dense material which is faintly linear at the narrowest part. The narrowest part is about 150 Å wide. The dense area in the surrounding cytoplasm associated with the narrowest part of the junction can be seen. (×114,000.)

FIG. 16.—A junction between two endothelial cells. At x can be seen a Y-shaped structure. This may represent the fusion of the outer leaflets of the apposing unit membranes. This area is a "tight junction." (×132,000.) I am indebted to Miss G. I. Schoefl for this micrograph.

FIG. 17.—A capillary from the pancreas. The endothelial cells are of varying thickness and at certain points (x) are very tenuous. A fibroblast (Fi) is in the space between the capillary and the surrounding acinar cells. (×7,000.)

FIG. 18.—Endothelial cell from pancreas to show that it contains the usual caveolae intracellulares and associated vesicles as well as extremely thin areas (x). The lumen contains many ferritin molecules, but not many have escaped into the surrounding tissue spaces. (×23,000.)

FIG. 19.—A "pore" in a pancreatic endothelial cell. The unit membrane of the cell appears to be continued across the "pore." (×170,000.)

FIG. 20.—A pore in a pancreatic endothelial cell. Ferritin in the blood has not escaped through the pore in 10 minutes. (×74,000.)

FIG. 21.—A pore in a pancreatic endothelial cell. Some ferritin is present in the basement membrane and beyond, but there is no clear evidence that it has reached this position by coming through a pore. The amount outside is much less than that in the lumen. (×74,000.)

FIG. 22.—Capillaries bordering an alveolus of lung. The very tenuous nature of the endothelium can be seen. It consists of a thin layer of cytoplasm sandwiched between two unit membranes. The thick basement membrane is shared with the epithelial cells lining the alveolus. The endothelium of the capillaries also contains thicker areas with caveolae. A small portion of such an area is at the extreme right of the lower endothelial cell. Ferritin is within the lumen of the capillaries. (×49,500.)

FIG. 23.—Cerebral capillary showing a closely applied adventitial cell around which the basement membrane of the capillary is split. The capillary is closely invested by neuroglia (NG). (×27,000.)

FIG. 24.—A portion of the wall of a cerebral capillary showing an almost total absence of caveolae intracellulares. One of the external surface is marked at x. (×32,000.)

FIG. 25.—Endothelial cells lining a liver sinusoid. The very tenuous cytoplasm rests on the villi of the underlying epithelial cells in the space of Disse. (×12,000.)

FIG. 26.—Liver. A Kupffer cell rich in organelles is continuous with the thin cytoplasmic lining over the villi of the epithelial cells. The very tenuous nature of the endothelium can be seen in the wall opposite the Kupffer cell. (×12,000.)

FIG. 27.—Saccharated iron oxide was injected intravenously into a mouse. Particles of saccharated iron oxide are entering caveolae intracellulares from the lumen of a cardiac capillary. It is seen in apparently detached vesicles and in one caveola abutting on the basement membrane. (×85,500.)

FIG. 28.—Ferritin molecules contained in fluid perfusing an isolated rat heart have entered the caveolae and vesicles, the concentration being greater in those nearest the lumen. Some ferritin is found in the basement membrane. (×47,000.)

FIG. 29.—Particles of saccharated iron oxide contained in fluid perfusing an isolated rat heart have entered the caveolae on the laminal surface and are seen also in those on the external surface and in the basement membrane. (×54,000.)

FIG. 30.—Capillary in pancreas. The vessel is surrounded by a basement membrane which is intimately associated with collagen (Col) fibres which are oriented concentrically to the capillary. (×32,000.)

FIG. 31.—Endothelial cell of a cardiac capillary. This shows the double nature of the basement membrane, which is composed of a lighter part adjoining the external membrane of the cell and a parallel darker part. It is sometimes said that the basement membrane is fibrillary. While this appears to be so, the appearance may be a fixation artifact, as the coagulated plasma in the lumen and on the surface of the endothelial cell does not look strikingly different from the basement membrane. (×82,500.)

FIG. 32.—Junction between two endothelial cells of a capillary from a heart perfused with fluid containing saccharated iron oxide. Particles have entered superficial caveolae and have penetrated partly through the intercellular junction. They have been stopped at the narrowest part, which is apparently "tight" to particles of this size. (×128,000.)

FIG. 33.—Junctions between endothelial cells of a capillary from a perfused heart. The perfusion fluid contained dinitrophenol and particles of saccharated iron oxide. The metabolic inhibitor, like a variety of other materials perfused, has made the junctions permeable to the particles but they have not come grossly apart. It is difficult to see particles in the "tight" portions. They are abundant in the basement membrane. (×74,000.)

FIG. 34.—From a glomerular capillary of a mouse which had been injected intravenously with ferritin. The ferritin molecules are escaping freely through the pores and are abundant in the basement membrane. (×47,000.)

FIG. 35.—From a capillary of the colon of a mouse which had been injected intravenously with ferritin. No ferritin molecules have penetrated the "pores" although they are abundant in the lumen of the vessel. At the arrow it appears that a caveola completely traverses the cell and is open at both ends; it does not, however, contain particles. (×99,000.)

FIG. 36.—Junction between two endothelial cells from the heart of a mouse injected with ferritin. Although ferritin is abundant in the lumen, none has penetrated into the junction. (×31,500.)

Figs. 2, 6, 11, 15, 27, 28, 29, 32, 33, and 36 are reproduced by permission of the Royal Society.

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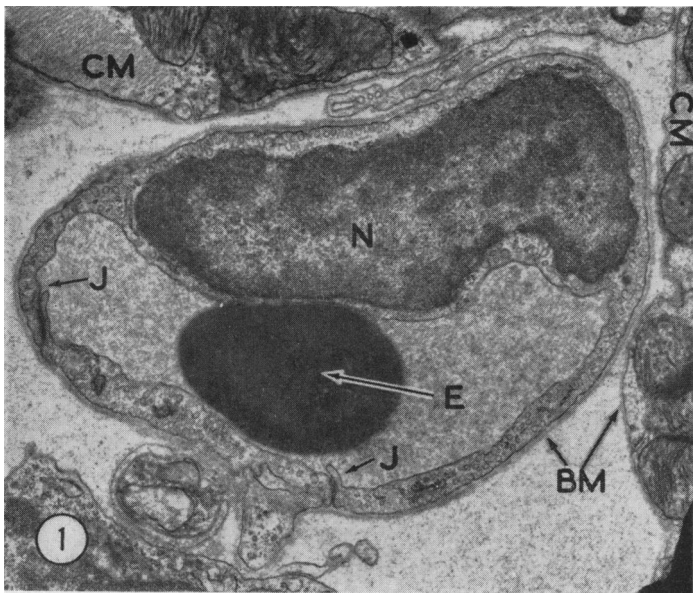
Abbreviations used in Figures

AC = Adventitial cell.
AL = Alveolar lumen.
BM = Basement membrane.
C = Centriole.
CI = Caveola intracellularis.
CM = Cardiac muscle.
D = Space of Disse.
DB = Dense body.
E = Erythrocyte.
En = Endothelial cell.
Ep = Epithelial cell.
F = Ferritin.
GB = Golgi body.
J = Intercellular junction.
L = Lumen.
MV = Multivesicular body.
N = Nucleus.
WP = Weibel-Palade body.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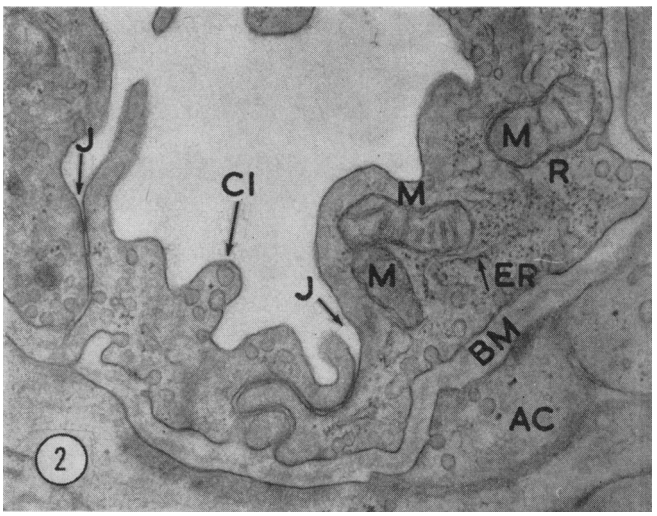
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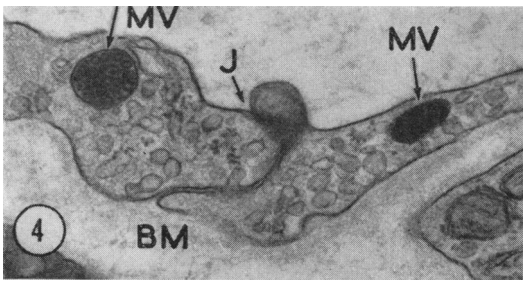
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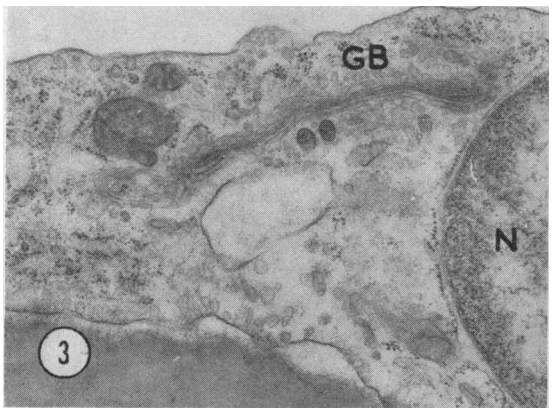
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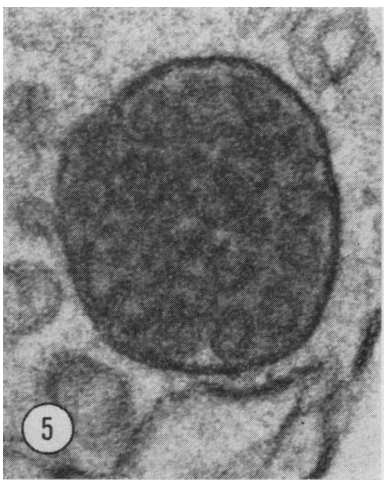
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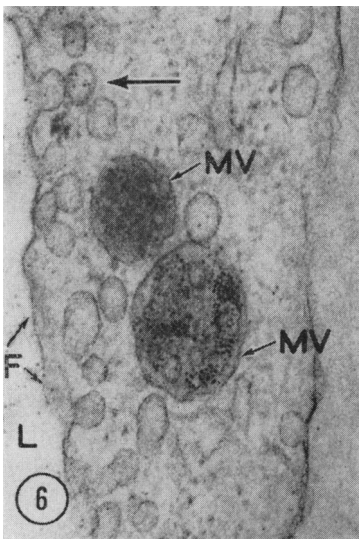
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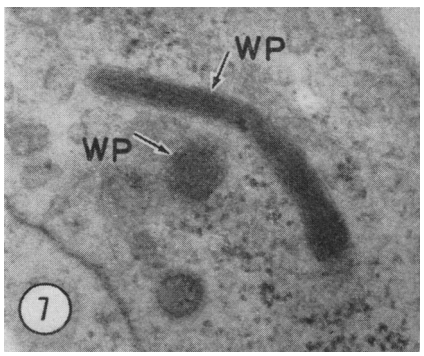
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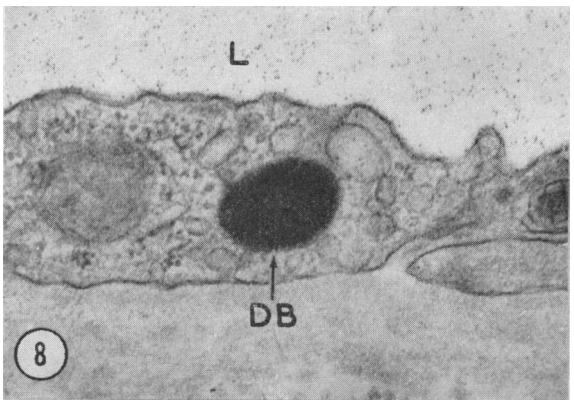
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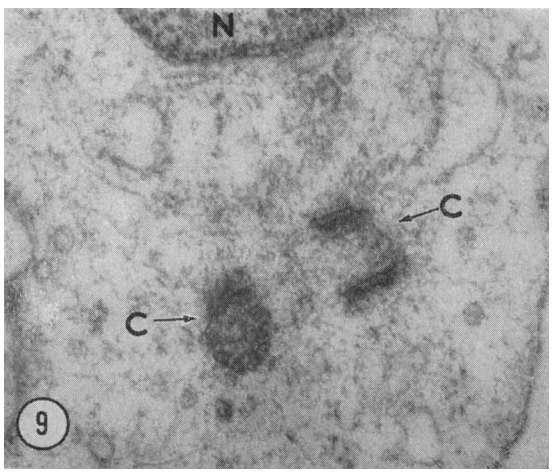
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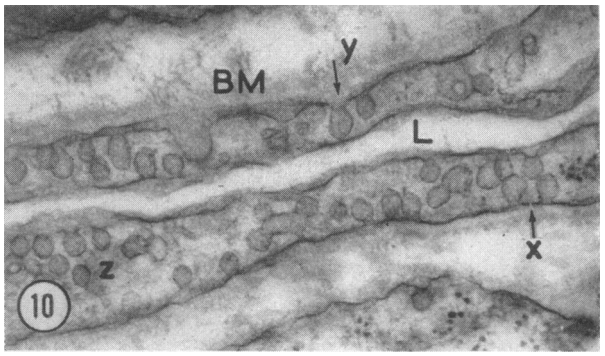
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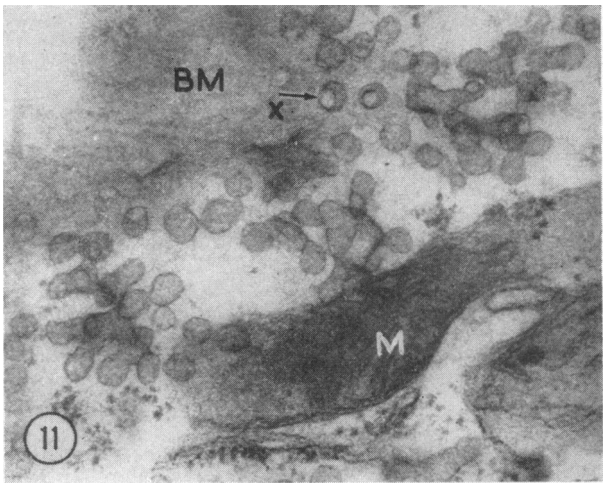
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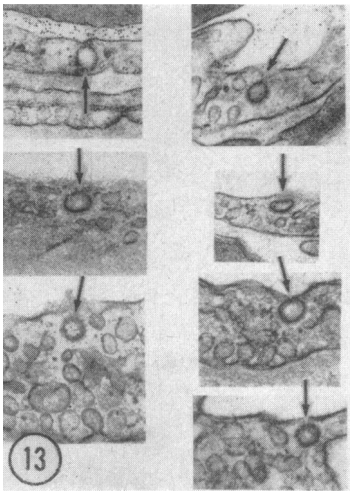
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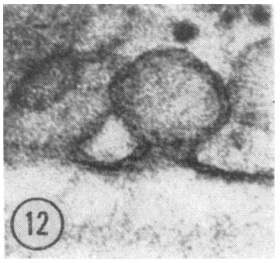
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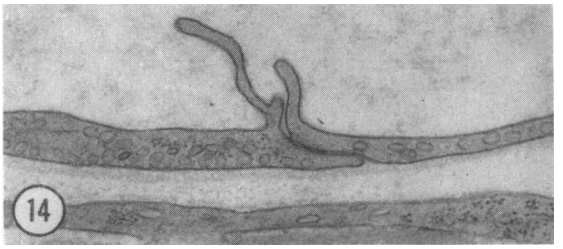
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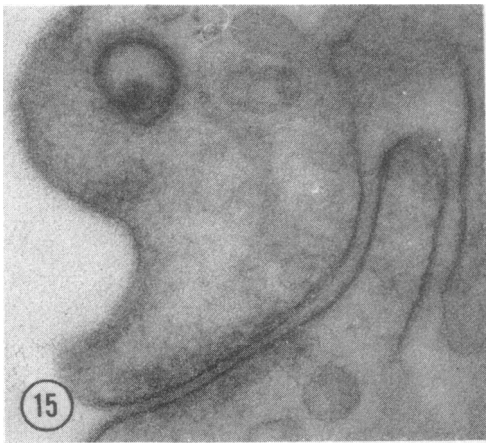
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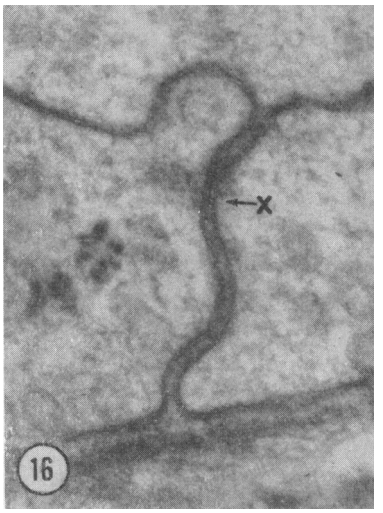
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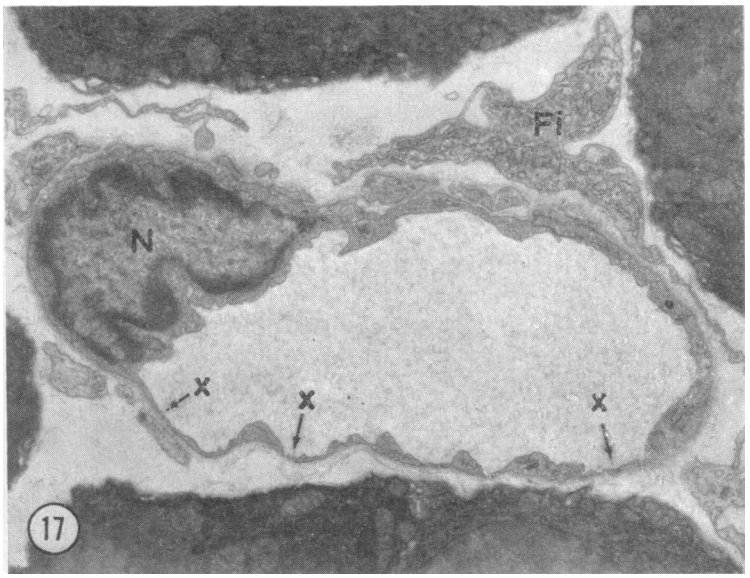
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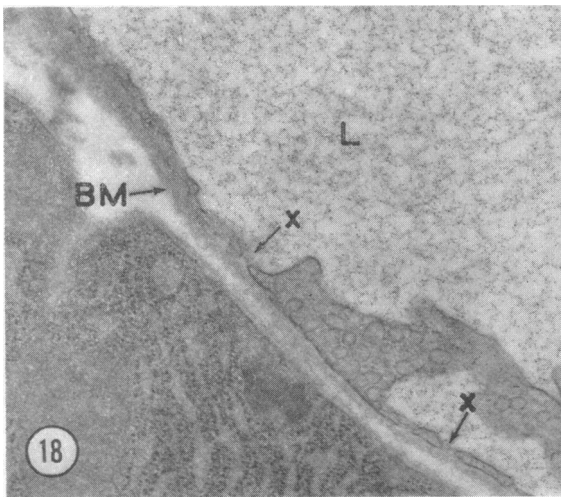
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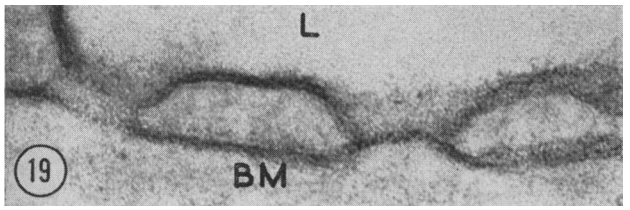
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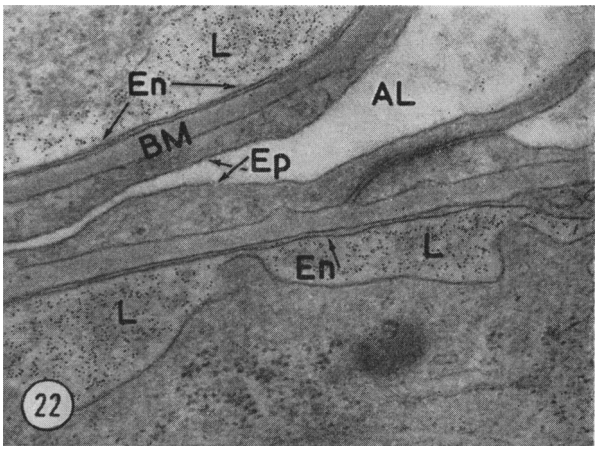
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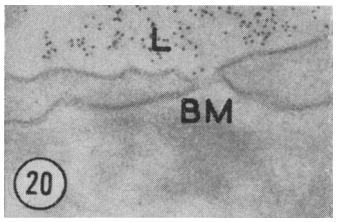
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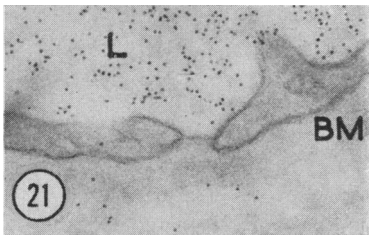
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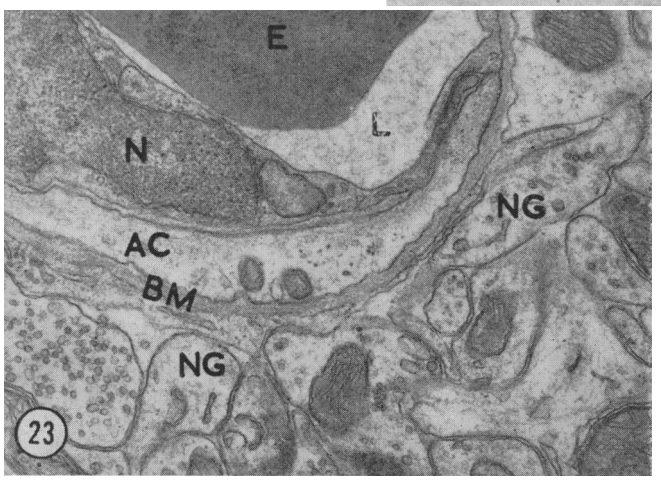
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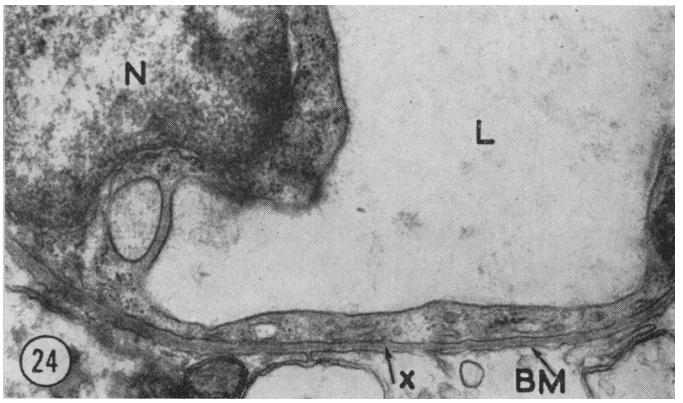
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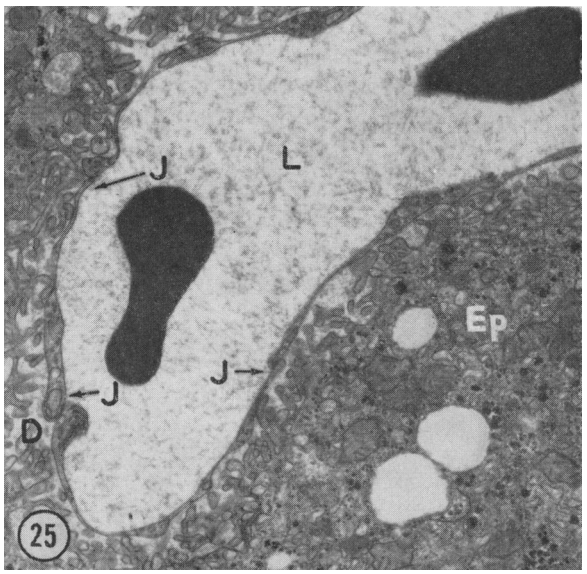
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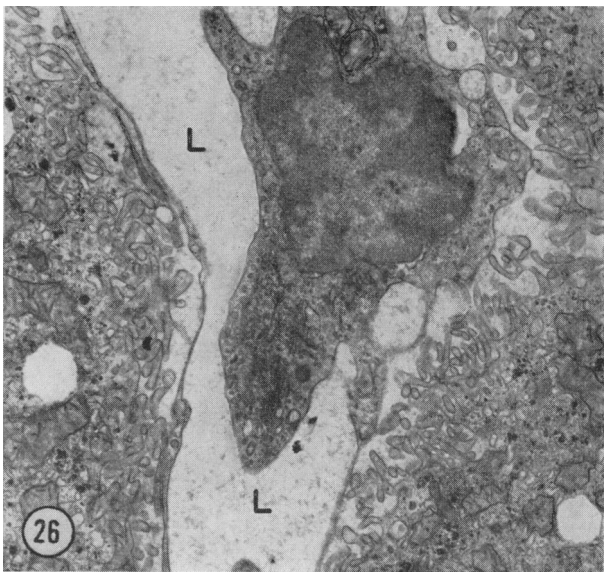
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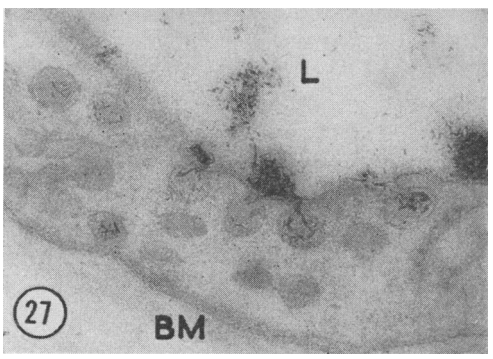
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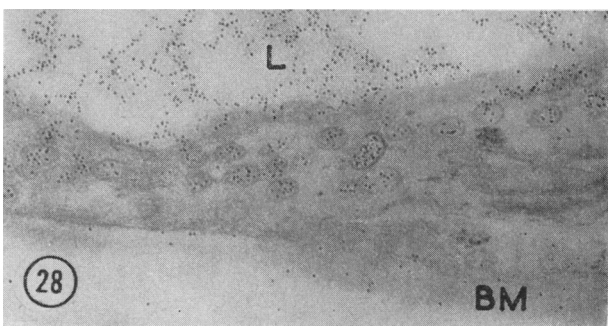
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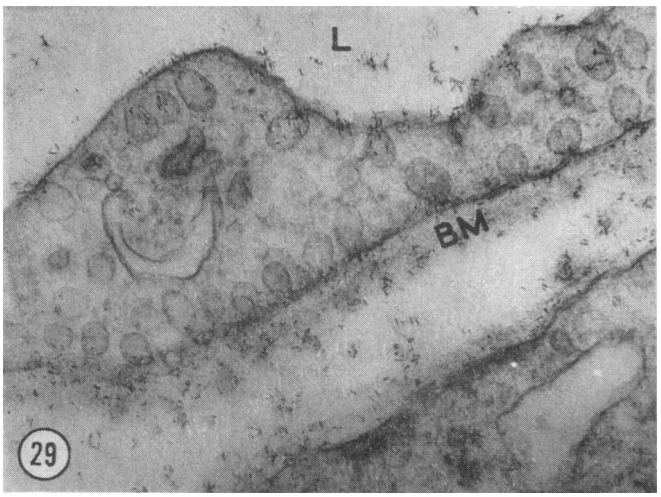
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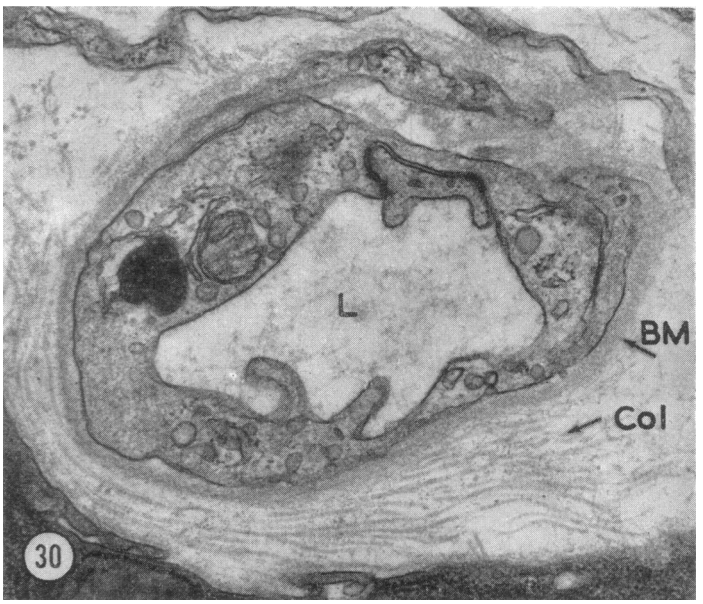
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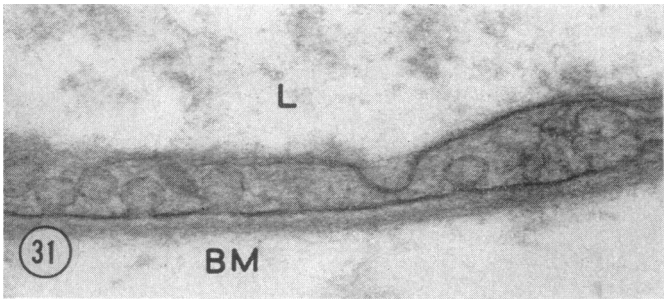
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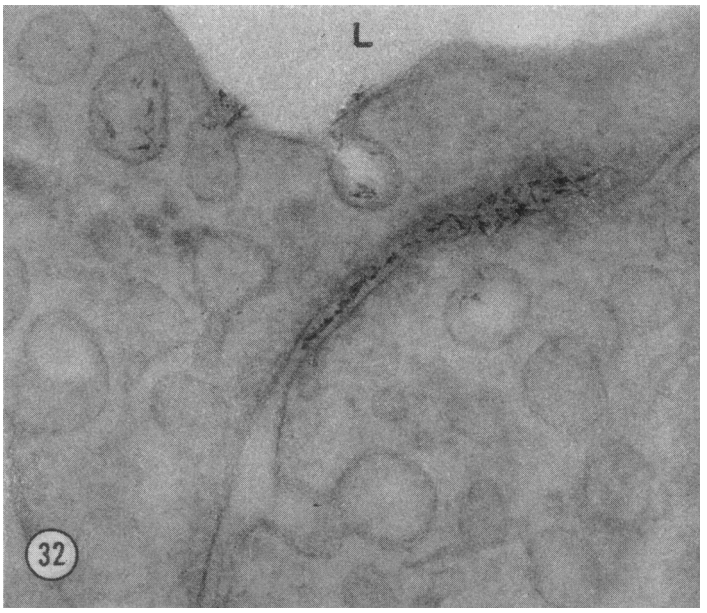
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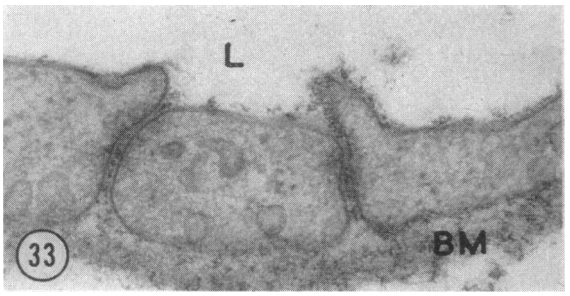
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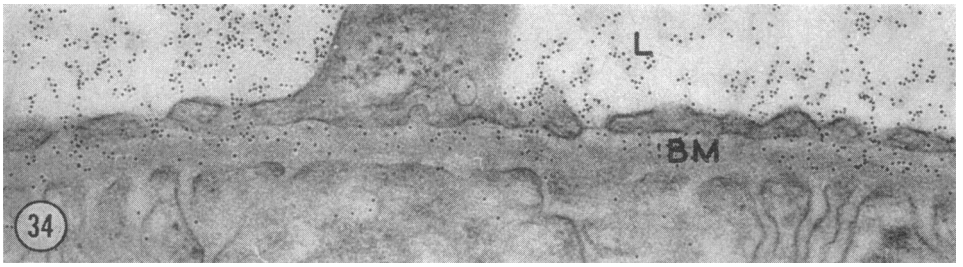
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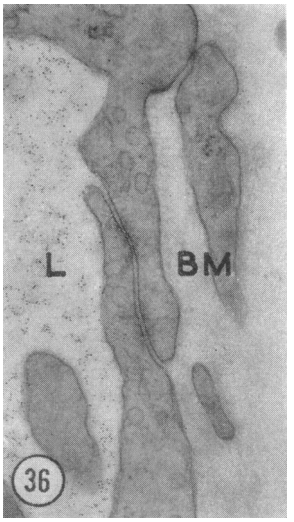
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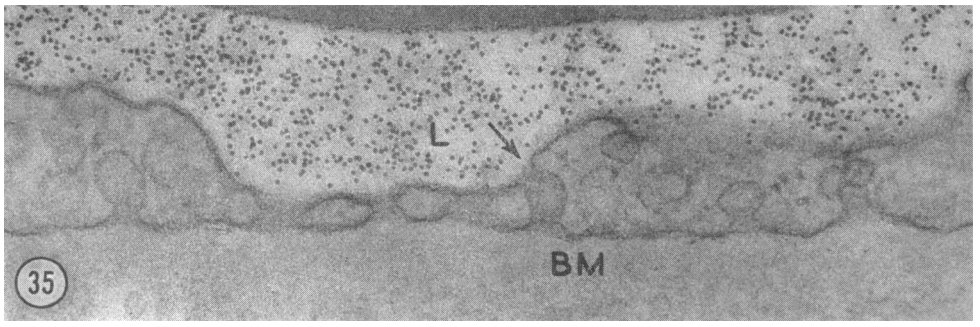
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within the capacity of the electron microscope, but so far nothing which can be identified as pores leading from the lumen to the outside of the cell has been clearly recognized. Palade, on morphological grounds, proposed that the caveolae intracellulares and vesicles play an important part in transferring fluid from the lumen to the outside of the capillary, and possibly vice versa. On this theory it is supposed that the plasma membrane of the luminal surface of the endothelial cell infolds, at the same time engulfing plasma from the lumen.

The caveola intracellularis thus formed is pinched off after reaching a certain size and passes as a vesicle across the cytoplasm to the outer surface, where the reverse process takes place and the plasma quantum is discharged on to the basement membrane. This idea has led to the postulate that the basement membrane and not the endothelial cell is the area in which the semipermeable qualities of capillaries really reside. This view on vesicular transport has been tested experimentally by Palade and others, using particles which are visible in the electron microscope. These test particles are of a considerable size, among the commonest being colloidal gold, ferritin which has a diameter of about 100 Å, and saccharated iron oxide, the particles of which may have a diameter of as little as 50 Å. Nevertheless, all these particles are of such a size that when we observe them we are observing the possible route of transport of large molecules from blood to tissues. It has been found that, after the injection of ferritin in sufficient quantity intravenously, molecules of ferritin are present in the vesicles, though not in my experience in very large numbers.

Heart-Lung Preparation of Simpson-Morgan

A very elegant preparation of Simpson-Morgan's can be used to investigate this point. He has devised a heart–lung preparation in the rat by means of which blood is pumped by the heart to be aerated in the lungs and thence returned to the heart, all other organs being excluded. It is possible to inject into this heart–lung preparation a solution of ferritin, so that the plasma contains a high concentration of ferritin molecules which does not diminish significantly during the course of the experiment because the reticuloendothelial system has been excluded. Even after two hours there is little ferritin in the extravascular tissues, nor is it common to find many ferritin molecules in the vesicles.

I emphasize that these experiments are conducted for two hours, and so I think it is difficult to conceive that these vesicles are being rapidly formed and that they are taking in gulps of whole plasma ; for, if they were, there should be far more ferritin particles within them and in the perivascular spaces than appears to be the case in these experiments. Nevertheless, if saccharated iron oxide is given intravenously into a mouse it is possible to find pictures which could be readily interpreted as being due to the passage of particles via the vesicular system (Fig. 27).

Attempts have been made to explore the possibility of vesicular transport by using the perfused heart of the rat. It is possible to keep rats' hearts beating for hours when perfused with saline solutions, and it is also possible to add marker particles to the perfusion fluids.

In such preparations ferritin can be found within caveolae intracellulares and vesicles at practically the same concentration as it is in the lumen of the vessel (Fig. 28). Ferritin particles under similar conditions of perfusion have also been found in the basement membrane and surrounding tissue spaces. The same can be shown to be true of particles of saccharated iron oxide (Fig. 29).

There is thus some not inconsiderable evidence that under conditions of perfusion marker particles of relatively large dimensions can enter vesicles, and a series of pictures can be constructed consonant with the hypothesis that vesicles move from the lumen across the endothelium to discharge their contents beneath the basement membrane. Such observations led to attempts to see how tough the vesicles were and whether any procedures demonstrably influenced their formation. It can be stated briefly that nothing so far tried seems to inhibit their formation. Lack of oxygen, metabolic inhibitors of one sort or another, cold, and the application of enzymes seem to leave the vesicles more or less unaffected or, at least, not destroyed.

The conclusion could be reached that the vesicles are either semi-permanent structures or are formed very slowly and are very resistant, and, if this is so, it is doubtful whether they are very actively ferrying plasma from one side of the cell to the other, at least when tracers are present in the plasma under conditions of experiment. It will have been noticed that there is a difference between the behaviour of the caveolae and vesicles when plasma is present and when it is absent. When plasma is present ferritin in particular does not reach the same concentration in the vesicles as in the lumen of the vessel. The precise reason for this still escapes recognition, and experiments are being pursued to see if this discrepancy can be resolved. It is possible that in plasma the particles are larger than they appear to be in electron micrographs because of the adsorption of plasma proteins.

Behaviour of Intercellular Junctions

Recently there has been renewed interest in the behaviour of the intercellular junctions because it is now suggested that it is in the region of the " tight junction " that the pores demanded by physiologists may exist.

Particles of saccharated iron oxide can be found in the intercellular junctions of capillaries of perfused hearts, but usually they do not pass completely down the junction. When they come to the narrow part—that is, the area generally regarded as a tight junction—they are stopped, at least for a time (Fig. 32).

That tight junctions may be not so " tight " under some conditions is shown in certain other experiments. In perfusion experiments designed to test the effects of such unnatural conditions as the presence of metabolic inhibitors or enzymes on the vesicular system and the intercellular junctions it was found that saccharated iron oxide was sometimes present throughout the junction. When dinitrophenol, for example, was added to the perfusion fluid particles of saccharated iron oxide were found along the whole junction, although the junction itself had not come apart in any gross fashion (Fig. 33). Fig. 33 shows that although the saccharated iron oxide appears to be present throughout the length of the junction it is very difficult to see particles in the narrow zone. Nevertheless, particles seem to be entering from the lumen and emerging on the exterior. There are certainly large numbers of particles in the basement membrane.

It is possible that small changes can take place in junctions, so that areas that are " tight " become less " tight," although the junction does not open grossly, and indeed still preserves the construction characteristic of a " tight " junction. This certainly appears to be the case under experimental conditions. It could well be true in certain physiological and pathological states. In inflammation and under the action of certain vasoactive substances junctions open widely. Such is not the case in the perfusion experiments.

Under the conditions of perfusion particles of saccharated iron oxide appear in the junctions, and, if saccharated iron oxide is injected intravenously, it is possible on rare occasions to see it in a junction, where it may pass the narrow or " tight " portion. On the other hand, molecules of ferritin are not generally found in the junctions in perfused hearts nor in

junctions either in intact mice injected with ferritin (Fig. 36) or in Simpson-Morgan preparations.

Karnovsky's Work

The type of investigation involving markers has been recently taken a stage further by Morris Karnovsky, and I am indebted to him for allowing me to mention his work, which has not yet been fully published. Karnovsky injected intravenously the protein peroxidase which has a molecular weight of 40,000 —that is, much less than that of the particles I have mentioned. He then did a histochemical reaction for peroxidase which detected the positions which the molecules of peroxidase had reached. The electron donor for the peroxidase reaction was 3-3-diaminobenzidine, the oxidized form of which reacts with osmium tetroxide to yield an electron-opaque final reaction product at the site of enzymatic activity. One minute after intravenous injection peroxidase was found in the vesicles lining the lumen of endothelium in cardiac capillaries and about halfway down the intercellular junctions. After three minutes the peroxidase molecule had completely passed through the intercellular junctions and it also appeared in the caveolae on both sides of the endothelium and in the basement membrane. It would seem that peroxidase molecules were passing very rapidly from the inside to the outside of the capillary wall. The deduction seems to be justified that there is a passage for molecules of at least the size of peroxidase both via the intercellular junctions and via the vesicular system. However, controversy on these matters is still at its height, and the final solution will depend on the elaboration of ever-better technical procedures and the taking of ever-better electron micrographs. And that is where we will have to leave this matter for the time being.

Pores

Some vessels, such as those of the glomeruli, have pores in them which can be demonstrated relatively easily to give passage to ferritin molecules (Fig. 34).

There are also pores in the vessels of the adrenal cortex that readily pass ferritin, but in other situations pores which superficially look very similar to those in the kidney and adrenal are not permeable to ferritin particles, or at least are not freely permeable to them. Such, for example, are those in the pancreas and colon (Figs. 17, 18, 19, 20, 21, and 35).

So there are pores and pores; those of the kidney freely let through quite large molecules, those of the pancreas and the colon do not appear to do so. Only further very refined research can determine what is the essential difference between these structures in the kidney and adrenal and those in the pancreas and intestine.

The Basement Membrane

The basement membrane was at one time thought to interpose a substantial impediment to the passage of molecules such as ferritin —that is, is was thought to be a filter. This is evidently the case in the capillaries of the glomeruli of the kidneys. Nevertheless, it is certain that ferritin molecules pass through the basement membrane in other situations relatively easily, although particles of saccharated iron oxide are impeded and tend to stay in it. Whether it has a fibrous structure permeated by other material is not certain. Perhaps it is a gel composed of mucopolysaccharides; perhaps it may be related to collagen. More research is needed to establish whether the basement membrane is purely a support for endothelial cells or whether it plays a significant part in conferring semipermeable qualities on blood capillaries.

Conclusion

In the time available I have been able to show you a little of the current knowledge of the morphology of cells which fifteen years ago were thought to form little more than a sheet of nucleated cellophane. The interpretation of the morphological appearances and an estimate of the importance, in terms of physiology and pathology, of the structures I have been able to show leaves much to be desired. As I have already said, there are no reasons for believing that the organelles, such as the mitochondria, endoplasmic reticulum and ribosomes, Golgi body, and nucleus, perform different functions from those performed by them in other cells.

The caveolae intracellulares, which are prominent in some but not all endothelium, may facilitate the passage of substances through endothelium even if they function only by furnishing a greater area through which diffusion may occur. The fine structure of the interendothelial junctions may hold the key to understanding how small quantities of plasma proteins may leak out of even the most impermeable blood-vessels.

From the point of view of pathology the fact that all endothelial cells so far examined seem to be able to take up particles of molecular size may be of some importance when considering the behaviour of virus particles, or indeed of any foreign protein particles which enter the blood-stream. The structure of the basement membrane may be of great importance not only physiologically but pathologically, as changes have been demonstrated to occur in it in diabetes and in glomerular nephritis. Endothelial cells are much involved in the development of the phenomena of inflammation, and it seems to be established that the great leakage of plasma during acute inflammation is due to the substantial separation of one endothelial cell from another at the interendothelial junctions and not to increased activity of the caveolae.

It is possible, too, that consideration of endothelial permeability may be of importance in elucidating the initial phases of the development of atherosclerosis. Indeed, it was a consideration of this which called my attention to the desirability of joining those who are investigating endothelium by modern methods.

Perhaps you will be kind enough to look on what I have said today as one more interim report on endothelium. Our knowledge is still far from being definitive, and I should expect to see the next ten years yield a rich harvest of new knowledge about the cells which stand between the blood and lymph streams and the cells of the tissue. I would expect to see exemplified the dicta that the introduction of a new technique is certain to be followed by new discoveries and that the pushing of a known technique to greater heights of technical achievement will produce new accretions of knowledge.

While thinking about what I've just said it occurred to me that I have somewhat unwittingly revealed that I do not think there is any sharp line of distinction between physiology and experimental pathology.

Those who wish to read a recent extensive account of the properties of vascular endothelium are referred to the article by G. Majno, "Ultrastructure of the Vascular Membrane," in the *Handbook of Physiology*, Section 2, Circulation vol. III, p. 2293, edited by W. F. Hamilton and P. Dow. American Physiological Society, Washington, D.C. 1965.